

ANNUAL REPORT FOR CALENDAR YEAR 2015
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National Clonal Germplasm Repository Staff

Permanent & Term Federal Staff

Nahla Bassil, Geneticist – Plants
Jill Bushakra, Research Associate, Genetics
Jeanine DeNoma, Bio. Science Tech., TC
Missy Fix, Bio. Science Tech., Distribution
Kim Hummer, Research Leader/Curator
Robert Larson, Ag. Sci. Tech/Field
Kyle Nemes, Program Support Assistant
April Nyberg, Bio. Science Tech., Genetics
Jim Oliphant, Bio. Science Tech., GH Manager
Yvonne Pedersen, Program Support Assistant
Kyle Nemes, Program Support Assistant
Joseph Postman, Plant Pathologist/Curator
Barbara Reed, Research Plant Physiologist
Joe Snead, Ag Science Tech/Field Manager



*Vietnam-US Plant collecting expedition Fall 2015.
L-R Kim Hummer, Tran Hoai, Jim Oliphant, Kien
Nguyen, at Phja Oac-Phja Den, Vietnam.*

Temporary Staff & Students

Katie Armes Carter, OSU/Mol. Lab Tech
Samantha Tochen, OSU Research Assistant
Jack Brennan, GH Pathways Student
James Dociamore, GH Pathways Student
Barbara Fick, GH Bio. Sci. Tech
Spencer Gilbride, IT Pathways Student
Sunny Greene, Student Intern TC Lab
Debra Hawkes, Bio. Science Aid, Greenhouse
Angelina Nachorniy, Bio. Science Tech
Jane Olson, Bio. Science Tech, Greenhouse
Kasey Schaefer, Bio. Sci. Aid/Molecular
Tyler Young, BENCO
Mitchel Elstad, Work Study
Brad Greenburg, Work Study
Clayton Skillman, Bio. Science Aid/Field

Graduate Students & Visiting Scientists

Meleksen Akin, GRA, OSU, Hort., Turkey
Natalia Salinas, GRA, OSU, Hort, Ecuador
Sukalya Poothong, GRA, OSU, Hort. Thailand
Sugae Wada, Post Doc. OSU

Stakeholder/Service Accomplishments

- 12,532 accessions, 67 genera and 772 taxa of 672 species of temperate fruit, nut, and specialty crops were conserved.
- Obtained a total of 202 new accessions and 376 new inventory items in CY 2015.
- Received 1,037 order requests and shipped a record 9,720 items in CY 2015.
- Collaborated with NCGRP, Ft. Collins, CO, on cryopreservation protocols of dormant blueberry, hazelnut, pear, and currant.
- Trained visiting scientists from Turkey, China, and Kazakhstan.
- Participated on Governing Board for USDA National Clean Plant Network.
- Scientific editor of the proceedings for the ISHS IHC symposium on Rubus and Ribes.
- Provided tissue culture assistance to hop breeders for a hop mutation breeding project.
- Meristemmed heat-treated pear and hazelnut accessions and multiplied them for the virus elimination program.
- Determined worldwide DNA testing needs in members of the Rose family we maintain and how best to meet them.
- Trained a graduate student in developing an economical molecular system for distinguishing hazelnut cultivars.
- Trained a high school student recruited through the Apprenticeship in Science and Engineering Program in field and molecular evaluation of black raspberry plants.

Research Accomplishments

- Published the first high throughput 90K genotyping platform in strawberry an octoploid crop.
- Obtained 95 samples of Rubus and Vaccinium and 4 other genera from Vietnam.
- Reported on successful development of microsatellite markers to assess genetic diversity and phylogenetic relationships of clonal medlar (*Mespilus* sp.) accessions.
- Determined more precise northern distribution of *Fragaria cascadiensis* from Oregon.
- Completed raspberry in vitro rooting study for media optimization.
- Developed microsatellite and single nucleotide polymorphism (SNP) markers that were used to construct the first linkage map in tetraploid blueberry.
- Demonstrated the efficiency of using microsatellite markers for predicting perpetual flowering in strawberry plants.
- Developed the first linkage map of black raspberry with microsatellite and SNP markers.

Administrative Overview

Staffing Changes

Yvonne Pedersen, our Program Support Assistant, retired at the end of February 2015, and in November, 2015 Mr. Kyle Nemes was hired to fill this vacancy. Samantha Tochen was hired in May, 2016 to replace Farm Manager Joe Snead, who retired in March 2015. Please join us in welcoming Kyle and Samantha to the NCGR family.

We have also not yet been able to recruit to replace Plant Physiologist Dr. Barbara Reed, who retired in July 2015.

Our Corvallis Location Administrative Office has new permanent employees who are assisting NCGR needs. Jimmy Rankin is our new Administrative Officer. Huong Nguyen is our IT Specialist. Christy Clark is our new Financial Technician. Jeff Strang is our Safety Officer. Becky Sloop is Jimmy's Administrative Assistant. In addition, we have recruited a new location Facilities Operations Specialist, Pete Belisle. We welcome these new staff members to our Corvallis ARS team.

EEO/CR/Outreach

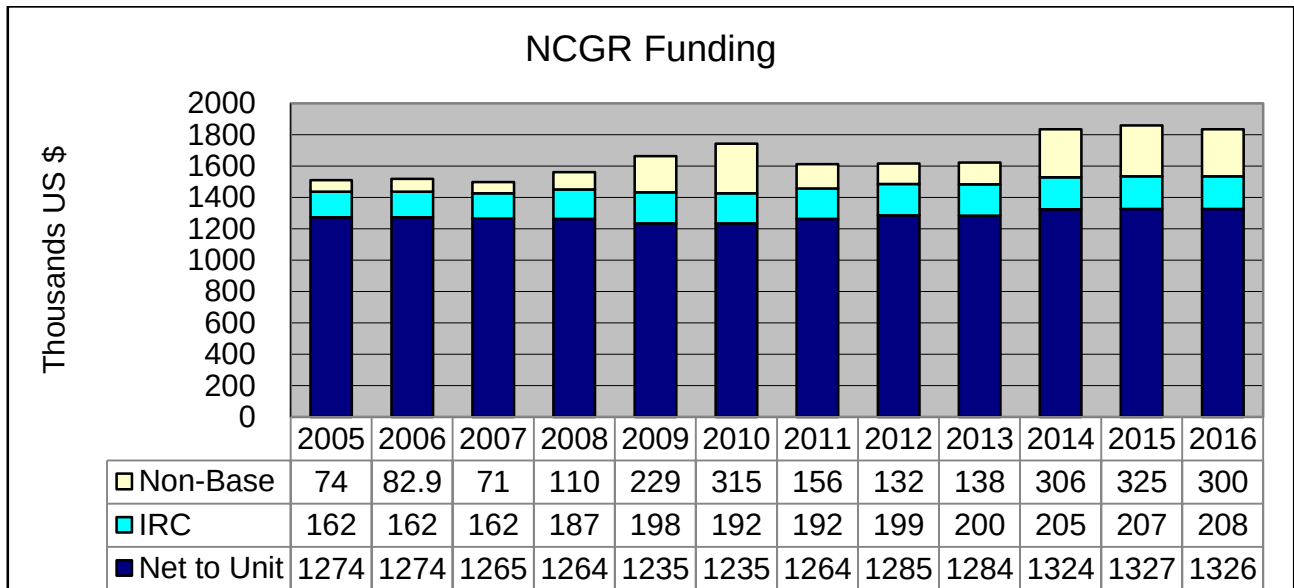
- A physically-challenged individual was trained in scanning technique and label preparation.
- A female horticulture student intern was trained for 9 months in tissue culture techniques.
- Through a Research Support Agreement with Oregon State University 3 female graduate students were trained.
- During the winter, 3 physically challenged high school students (program was funded through local school district grants) were trained in greenhouse management activities.
- 15 mentally or physically individuals from a local private organization (Work Unlimited) were trained in strawberry greenhouse activities.
- NCGR staff attended Fascination of Plants day; 2 job fairs, mentored high school students to improve job recruitment skills; and assisted Habitat for Humanity in repair of housing for economically challenged individuals.

Budget

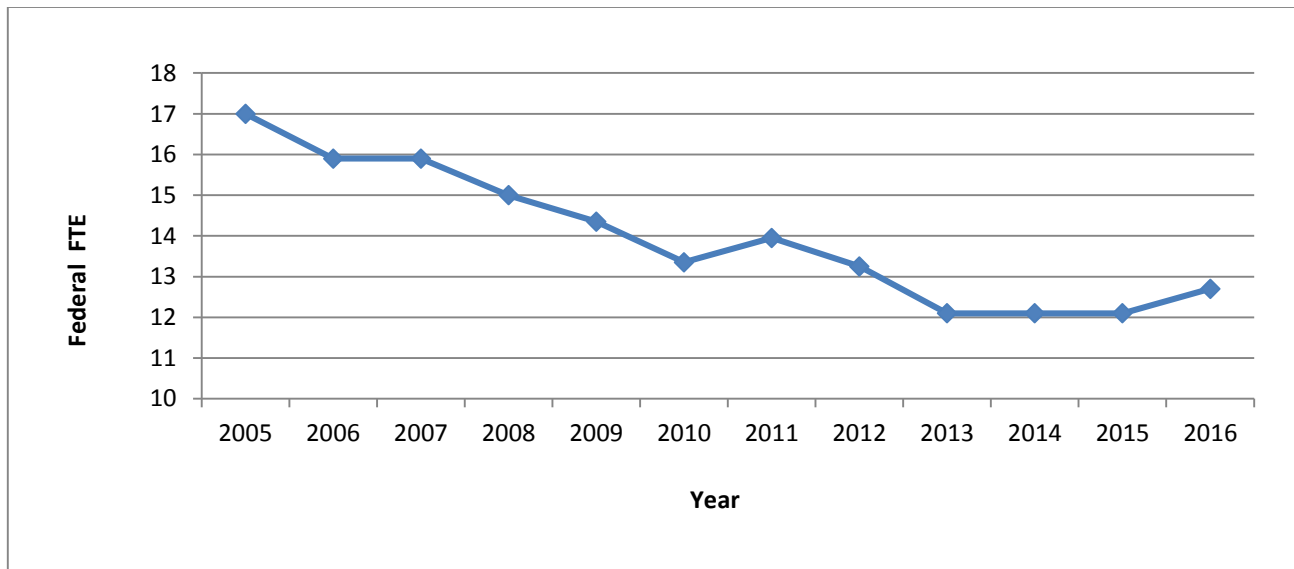
The CY 2015 year remained level over 2015. Our total federal budget is about \$1.54 million. The challenge for our fixed budget this year is that Corvallis was just designated to be added to Portland regional locality pay. This locality pay designation is equivalent to about 5 % more in pay for each of our employees. Unfortunately, with this pay increase our bottom line remains static. The effect is to reduce our operating expenses by the equivalent amount (roughly by \$50K). This will be an interesting year because this salary increase was not indicated when the annual budget was planned.

Our scientific staff is encouraged to seek soft funding from a wide variety of research granting opportunities to supplement our base federal funds. Our scientists have been successful with obtaining grants from commodity commissions and research consortium funding.

Budget History



Employees History



Non-base and Extramural Funding for 2015

Research Proposals

Funded	FY 2015		
California Pear Board	\$35,050	Pear rootstock chlorosis	Reed
OR Hazelnut Comm.	\$12,000	Hazelnut elite selection tissue culture	Reed
ISTC-ARS Kazakhstan	\$10,000	Pear and apricot tissue culture	Reed
USDA Plt. Exploration Off.	\$33,390	Vietnam Collection of Rubus/ Vaccinium	Hummer
SCRI subcontract	\$10,000	Hazelnut micropropagation	Reed
Ag Res. Foundation	\$12,500	Blueberry medium development	Reed
SCRI GRANT	\$127,822	Genomic infrastructure black raspberry	Bassil
SCRI Grant	\$40,003	RosBREED2	Bassil
NWCSFR	\$33,905	aphid resist in black raspberry/ Markers	Bassil
ARF	\$12,500	Aphid Resistance in Black Raspberry	Bassil
NARBGA	\$1,000	Labeling Blackberry Species	Hummer
USDA-APHIS Farm Bill	\$15,100	Survey of berry germplasm for viruses	Postman
USDA ARS NPGS	\$1,300	SWD on Fruit	Hummer
USDA-ARS-NPGS	\$14,000	Improve Pyrus SSR fingerprinting set	Postman, Bassil
Total	\$245,630		

Facilities

Security. We upgraded the data bridge to our North farm. Our staff has new smart cards for entering locked doors. A new multi-zone system was installed replacing a 30+ year old system. We installed a new stairway to a storage loft to replace a wooden ladder. We are working with Beltsville engineers for potential replacement of our aging screenhouses with a glass or twin-wall polycarbonate growing structure. If approved, construction is anticipated in 2016.

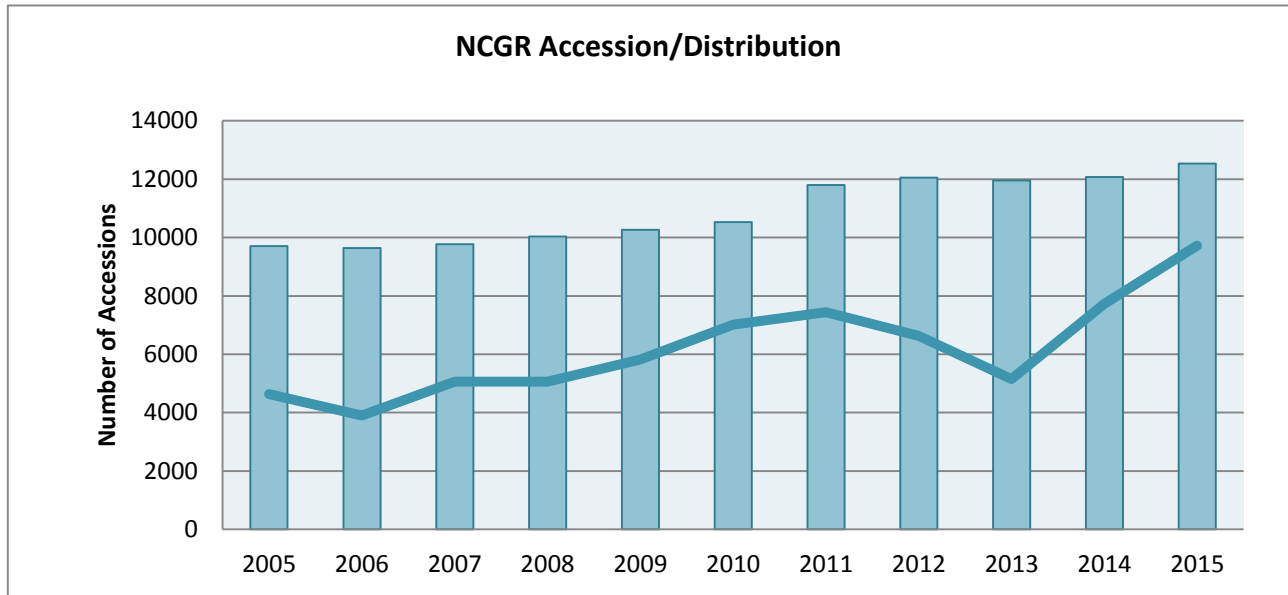
Visitors in 2015

By Kyle Nemes

Many visitors came through the Repository's front door during business hours. Guests arrived in large or small groups, as organized class tours or as individuals. About 25-30 people attended a blueberry open house in July. Groups such as the Oregon Processed Vegetable Committee used the Repository conference room for their annual meetings. Educational tours ranging from 2 to 150 individuals came from Willamette University, Home Orchard Society, Chemeketa and Linn-Benton Community Colleges, Oregon State University, and various garden clubs. Our ARS-Corvallis Outreach Diversity and Equal Opportunity Committee arranged a tour for 28 summer students to the three ARS Corvallis units. International visitors came from India, China, Japan, New Zealand, Ecuador, Spain, Turkey, Thailand, Australia, and Canada. We also hosted graduate students and visiting scholars from Thailand, Ecuador, and Turkey.

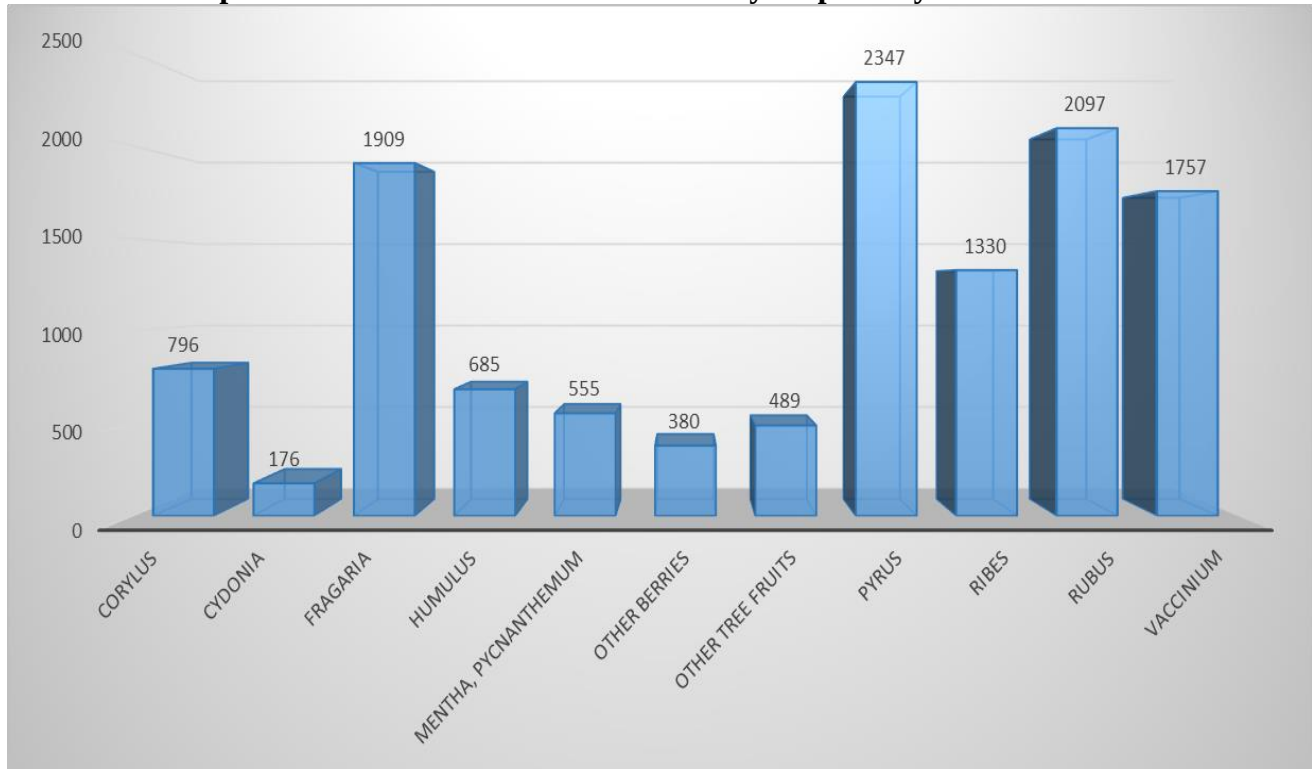
Germplasm Collections

Corvallis Germplasm Collections 2005 -2015



Bars represent number of accessions in the NCGR Collection. Line represents number of accessions distributed. We distributed 9,720 accessions in 2015, a record for our site!

Corvallis Germplasm Collections – Accession counts by crop – May 2016



Corvallis Germplasm Collections – Total seed and plant accessions by genus, May 2016.

<i>genus</i>	<i>count</i>
<i>Actinidia</i>	4
<i>Agapetes</i>	23
<i>Amelanchier</i>	49
<i>Amelasorbus</i>	1
<i>Arbutus</i>	3
<i>Aronia</i>	9
<i>Asimina</i>	40
<i>Buxus</i>	1
<i>Camellia</i>	1
<i>Castanea</i>	3
<i>Cavendishia</i>	5
<i>Ceanothus</i>	39
<i>Celtis</i>	1
<i>Chaenomeles</i>	20
<i>Cornus</i>	1
<i>Corylus</i>	796
<i>Crataegomespilus</i>	3
<i>Crataegosorbus</i>	1
<i>Crataegus</i>	27
<i>Crataemespilus</i>	1
<i>Cydonia</i>	176
<i>Cynodon</i>	5
<i>Dimorphanthera</i>	3
<i>Docynia</i>	1
<i>Empetrum</i>	17
<i>Epigaea</i>	1
<i>Fagus</i>	2
<i>Fragaria</i>	1909
<i>Gaultheria</i>	30
<i>Gaylussacia</i>	16
<i>Hippophae</i>	1
<i>Holodiscus</i>	4
<i>Humulus</i>	685
<i>Juglans</i>	29

<i>genus</i>	<i>count</i>
<i>Lonicera</i>	83
<i>Lycium</i>	14
<i>Macleania</i>	5
<i>Malus</i>	10
<i>Mentha</i>	460
<i>Mespilus</i>	60
<i>Micromeria</i>	1
<i>Peraphyllum</i>	8
<i>Pernettya</i>	1
<i>Physocarpus</i>	1
<i>Potentilla</i>	8
<i>Psammisia</i>	1
<i>Pseudoccydonia</i>	3
<i>Pycnanthemum</i>	95
<i>Pyracomeles</i>	1
<i>Pyronia</i>	7
<i>Pyrus</i>	2347
<i>Rhododendron</i>	6
<i>Rhodomyrtus</i>	1
<i>Ribes</i>	1330
<i>Rubus</i>	2097
<i>Sambucus</i>	171
<i>Schisandra</i>	10
<i>Sibbaldia</i>	2
<i>Sorbaria</i>	1
<i>Sorbaronia</i>	7
<i>Sorbocotoneaster</i>	3
<i>Sorbopyrus</i>	11
<i>Sorbus</i>	135
<i>Symphysia</i>	2
<i>Vaccinium</i>	1757
<i>Zelkova</i>	1
<i>Zoysia</i>	5
TOTAL	12,551

Corvallis Tree Collections 2015

Joseph Postman

Pears. January 1, 2016, the NCGR *Pyrus* collection included **2260 clonal pear accessions** and **355 seedlots** representing 36 *Pyrus* taxa from 59 countries. A collection of 225 clones are backed up *in vitro* as shoot cultures at 40° F, and 340 clones are backed up as small potted screenhouse trees. NCGR houses 976 European Cultivars, 179 Asian Cultivars, 119 Hybrid Cultivars, 170 Rootstock Selections, 36 Perry (cider) Cultivars and 916 trees representing pear wild relatives. Perry pears continue to be some of the most requested accessions, reflecting the surge of interest in hard cider in the U.S. Scionwood distribution was suspended in 2015 due to the detection of *Xylella fastidiosa* in several trees in the *Pyrus* field collection. A trial testing the effectiveness of hot water treatments to sanitize dormant scionwood from infected trees was initiated in Dec. 2015, with follow-up testing scheduled for July 2016.

Hazelnuts. The *Corylus* collection consists of 865 living trees including 458 cultivars/selections and 407 wild relative species trees. The hazelnut collection represents 20 *Corylus* taxa from 36 countries. Eastern Filbert Blight (*Anisogramma anomala*) was observed in more than a dozen trees in the *Corylus* field collection in 2015, making annual prophylactic fungicide sprays and pruning to remove infections essential. Propagation efforts continue in support of the establishment of field rows dedicated to the shrubby species *C. americana* and *C. heterophylla*, and to the re-location of various tree-hazel species to a separate plot near the pear field. We are also in the process of propagating the core collection (177 accessions) as self-rooted trees to replace the remote backup collection at the USDA genebank in Parlier, California.

Quince. The Corvallis genebank maintains 176 clonal accessions and 31 seedlots of *Cydonia* and the closely related genera *Docynia*, *Pseudocydonia*, *Pyronia*, and *Chaenomeles*. The various species of quince are represented by 9 taxa from more than 21 countries. The collection includes 16 rootstock clones, and 76 selections grown for their edible fruit. The quince collection has occasionally been afflicted by the bacterial disease Fire blight, requiring the removal of blighted shoots or branches.

Samantha Tochen applies a fungicide spray to prevent the spread of Eastern filbert blight in the NCGR hazelnut germplasm collection, April 2016.



Corvallis Field Collections

Samantha Tochen and Joseph Postman

The Corvallis genebank maintains 2,906 clonal accessions in field plots on 20 acres at the OSU Lewis Brown Farm, and 1,735 clonal accession in field plots on the 40 acre NCGR North Farm. A total of 4,641 Repository accessions are maintained in permanent field collections, representing 1,313 berry accessions and 3,328 tree accessions.

NCGR Permanent Field Collections by Genus

Location	Genus	Accessions
OSU Farm	Corylus	811
OSU Farm	Pyrus	1743
OSU Farm	Vaccinium	352
NCGR Farm	Actinidia	130
NCGR Farm	Juglans	34
NCGR Farm	Other Berry Crops *	101
NCGR Farm	Other Pome Fruits	343
NCGR Farm	Pyrus	397
NCGR Farm	Ribes	730
Total Field Accessions		4,641

*130 Actinidia, 30 Lonicera, 63 Sambucus, 8 Aronia

Corvallis Greenhouse/Screenhouse Collections

By Jim Oliphant

- Rearranged plants in 3 screen houses to give Fragaria cultivars the coolest house
- Replaced drip irrigation systems in 3 screen houses
- Begun modifications creating humid tropical conditions to maintain tender accessions
- Re-propagated the entire Raspberry collection and also build a new trellis system
- Re-propagated numerous Vaccinium accessions including the entire cranberry collection

Corvallis Seed Lab

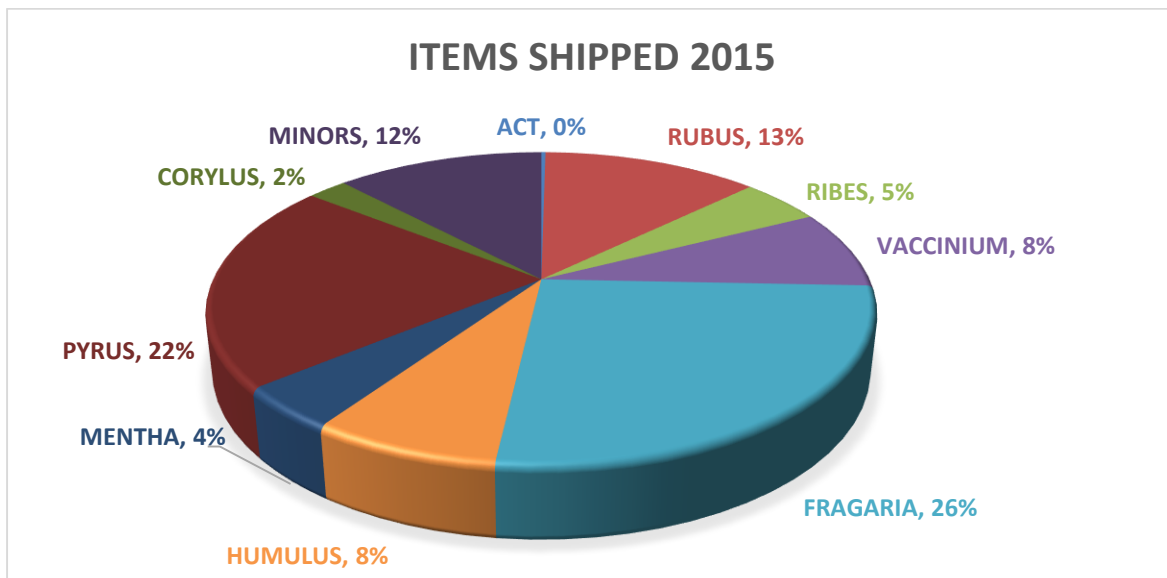
By Missy Fix

During CY 2015, 1741 seed items were shipped. 1647 were from the Major genera and 94 from the Minor genera. The most requested Genera was the Fragaria with 718 requests. Rubus was the next popular with 351 requests. Lonicera was the most requested in the minor genera with 34 requests. Sambucus was next with 23 requests. We received 42 new seed accessions – 13 Vaccinium, 7 Rubus, 13 Fragaria and 5 Pyrus 2 Humulus 1 Sambucus, and 1 Docynia. To help preserve our seed collection, Kim Hummer donated open pollinated seed of blackberry, yellow raspberry, red raspberry, blueberry, hops and strawberry; Joseph Postman donated pear seed to be sent out as educational seed for those teaching in elementary through High School or home school environment. These items have also been requested by community garden groups and various non-profit groups.

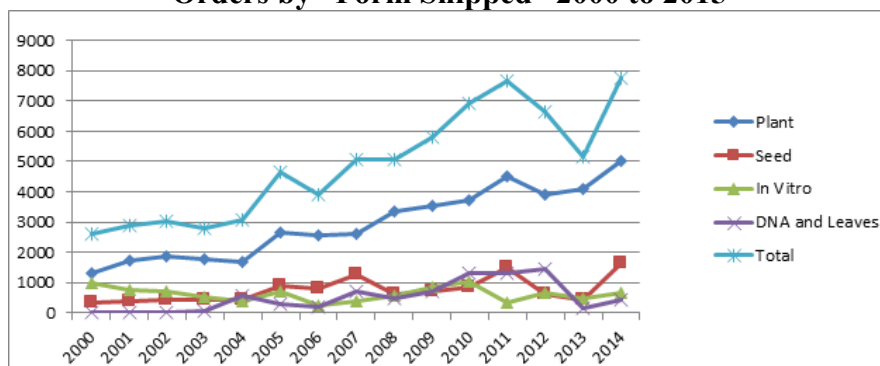
Distribution

by Kim Hummer and Missy Fix

- In CY 2015, NCGR staff shipped 9,720 items as seeds, cuttings, runners, scionwood, rooted plants, tissue cultures and DNA and leaf samples and informational material.
- In CY 2015, 658 new orders were received for small fruit and 146 new orders for scion or budwood. More than 864 orders were shipped.
- The pears and strawberries topped the list of crops distributed.
- Domestic individuals, state agencies and universities, and ARS researchers received the most germplasm from Corvallis in 2015.
- With the various educational systems such as grade schools, home schooling, and community gardening arenas requesting plant and or seed material, the addition of our educational seed has allowed us to fill orders that otherwise would have been cancelled. In all 536 seed packets were distributed.



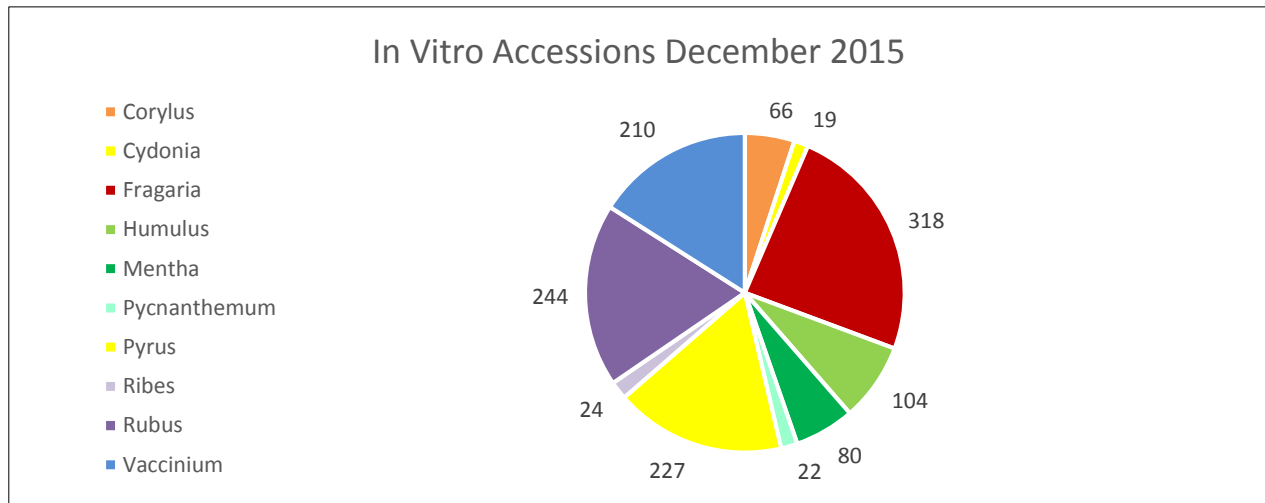
Orders by "Form Shipped" 2000 to 2015



Tissue Culture and Cryopreservation

By Jeanine DeNoma

The *in vitro* collection contains a subset of the collection held at the Repository. It includes primarily core accessions, those at risk outside the lab and accessions frequently requested. In December 2015, 1314 accessions were in culture, most in cold storage. Accessions in storage are evaluated every three to four months and are repropagated as needed.



During the 2015 spring, we emphasized collecting *Fragaria* accessions, initiating 115 new cultures from screen house plants that had recently been repotted and produced vigorous runners. We also initiated three *Corylus*, 10 *Humulus*, nine *Rubus* and 16 *Vaccinium* accessions. Most newly collected accessions have been tested, propagated and placed into cold storage. *Pyrus* and *Corylus* accessions regenerated from heat-treated meristems were propagated in preparation to be rooted in the greenhouse and retested for virus.

***Corylus* Culture.** Ph.D. student Meleksen Akin continued to improve the *in vitro* hazelnut growth medium. Studies were conducted on the response of hazelnuts to mineral nutrients including nitrogen ratios, increased nitrogen, MgSO_4 , and KH_2PO_4 . Additional analysis of both current and earlier data, using advanced data mining techniques, provided insight into the mineral nutrient requirements of hazelnuts with diverse genetic backgrounds. A final improved *Corylus* culture medium (Hazelnut 2015 Medium) based on these analyses is now being testing and will be released for public use in 2016.

***Raspberry* Culture.** Studies completed by recent Ph.D. Sukalya Poothong were published concerning the response raspberry cultivars to mineral nutrients. Increased amounts of the mesos stock solution (CaCl_2 , KH_2PO_4 and MgSO_4) and some changes in the nitrogen stocks produced excellent growth for the wide range of *Rubus* germplasm tested. These changes improved plant quality, multiplication and shoot length in all cultivars. We are now using this medium for the germplasm collection.

Research Collaborations. Collaborations include those with the OSU Horticulture Dept. Dr. Sugae Wada for pear, hops and hazelnut, ARS, Dr. Randal Niedz at Ft. Pierce, Florida for mineral nutrition studies, Dr. Maria Jenderek at Ft. Collins for cryopreservation, Mr. Anthony Shireman at Fall Creek Nursery for blueberry medium, the University of Nebraska and Nebraska Forestry Department for a *Corylus* SCRI micropropagation subcontract, and internationally with Kazakhstan for germplasm collection, tissue culture medium development and cryopreservation.

Germplasm Storage by Cryopreservation (Long-Term Storage). Long-term storage is coordinated with Dr. Maria Jenderek of the Vegetative Propagation Group at NCGRP from plant materials supplied by NCGR for back-up in liquid nitrogen.

Molecular Genetics

By Nahla V. Bassil

Graduate Students

Natalia Salinas completed her M.S. studies and graduated in August. PhD candidate, Meleksen Akin began her work on developing a robust fingerprinting set for hazelnut.



Genetics Lab Team joined by Jim Oliphant and Joseph Postman and collaborator Kelly Vining celebrating Natalia's graduation. Left to Right: Jim Oliphant, Nahla Bassil, Katie Carter, Natalia Salinas, April Nyberg, Jill Bushakra, Kacey Schaefer, Joseph Postman, and Kelly Vining.

Saturday Academy high-school summer intern Hanna Riley worked in the field and lab on the black raspberry project assessing the degree of virus infection in two field planted populations. She used the enzyme-linked immunosorbent assay (ELISA) method to detect the presence or absence of viral particles extracted from fresh leaf tissue.

Projects Completed in 2015

Developing genomic tools for blueberry. Construction of linkage maps in the tetraploid mapping population of ‘Draper’ x ‘Jewel’ was completed. In total, 233 simple sequence repeat (SSR) and 1,794 single nucleotide polymorphism (SNP) markers were analyzed and found to show segregation patterns consistent with a random chromosomal segregation model for meiosis in an autotetraploid. The ‘Draper’ map consisted of 12 linkage groups, associated with the haploid chromosome number for blueberry, and the ‘Jewel’ map had 20 linkage groups.

Testing markers associated with remontancy in strawberry. In collaboration with Daeil Kim, Jim Hancock, Chad Finn and Beatrice Denoyes, we evaluated five SSR markers linked to remontancy on LG IV in strawberry in multiple sources of remontancy in the RosBREED-evaluated germplasm. These strawberries were also phenotyped for this trait in at least two geographical locations (Corvallis, OR and East Lansing, MI). In segregating populations from MI and OR, approximately 17% of the progeny did not segregate based on pedigree indicating they were not true to type. In addition, discrepancies between different origins of parents, ‘Fort Laramie’ and FRA 1701 in the MI breeding programs and ‘Tillamook’ and ‘Puget Reliance’ in the OR program, were found suggesting different genotypes sharing the same name. This genomic region was highly associated with remontancy as alleles from each of the five SSRs were associated with remontancy in most sources. At the Bx215 SSR, the absence of 129, 123, 127 and 134 predicted once flowering (non-remontancy) in 100% of the germplasm where the original source of remontancy was that obtained from the *F. virginiana* subsp. *glauca* from the Wasatch Mountains through ‘Capitola’, ‘Seascape’ and ‘Tribute’.

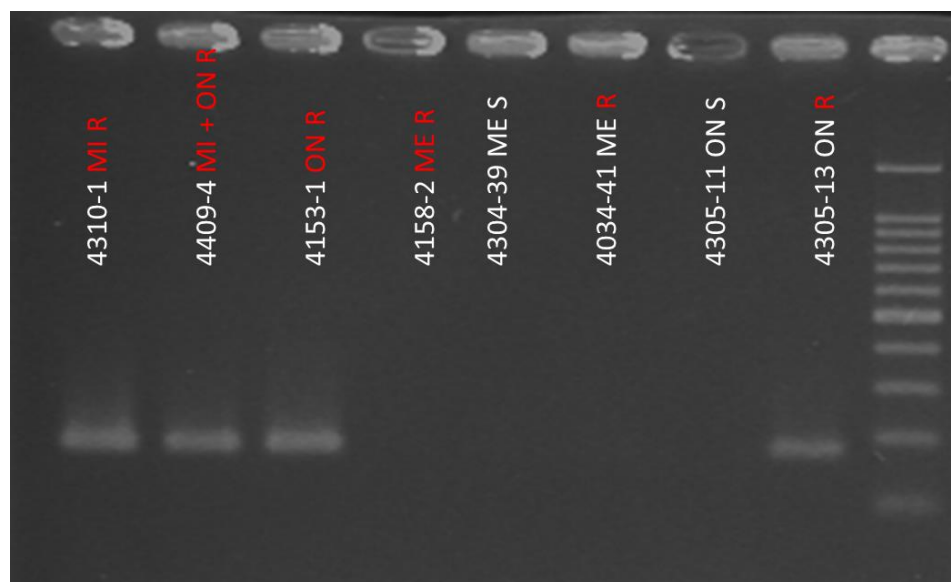
Testing markers associated with high soluble solids content in strawberry. This study evaluated the usefulness of a subgenome-specific SSR marker, EMFv006, in predicting SSC in a broad range of germplasm encompassing 893 worldwide cultivars, selections and breeding populations. Two alleles were consistently associated with low SSC: 209 and 215; and two alleles were associated with high SSC: 219 and 221. Plants with the 213:219 genotype always had higher SSC than those with the 213:209 or 213:215 genotypes in MI, OR and NH but not in CA. Heritability (H^2) was low (0.02-0.09) in MI and OR in 2011 and 2012 and in NH in 2011 indicating that this trait is highly influenced by environmental conditions. The proportion of phenotypic variance (PVE) explained by EMFv006 was high (0.65- 0.86) in comparison with those of H^2 in most states and both years indicating that most of the genotypic variance observed can be explained by this locus. Much of the variability seen in SSC in this study could be explained by differences in environments and fruit ripening at the time of harvest and their effects on SSC as this trait is sensitive to environmental factors such as temperature and harvest date. We believe that it may be necessary to develop a more standardized protocol of measuring SSC across states and years to obtain more reliable data for each individual. Most importantly, this study illustrates the usefulness of introgressing novel alleles from wild accessions into the cultivated gene pool. Both alleles consistently associated with high SSC, 219 and 221, originated from wild *F. virginiana* parents from the “supercore” and were not present in any of the *F. ×ananassa* cultivars genotyped in this study except for an old European selection, ‘Jucunda’, of unknown pedigree.

Black raspberry genomic resource development. The USDA-ARS NCGR manages and maintains a collection of over 175 black raspberry germplasm accessions, which includes newly collected wild accessions from 130 locations across 27 U.S. states and two Canadian provinces. Evaluation of this wild germplasm led to the identification of three potential sources of aphid resistance, and potential new sources of resistance to the fungal pathogen *Verticillium dahliae*. We are building the genomic infrastructure for black raspberry by developing, and making available, genomic tools including molecular markers for construction of linkage and physical maps, and a draft genome assembly that will benefit both black and red raspberry breeding programs across the U.S. In 2015, we constructed a genetic linkage map consisting of seven linkage groups representing the seven haploid chromosomes of black raspberry which includes a morphological locus for aphid resistance on group 6. A draft genome assembly of 240 megabase pairs (Mbp) was also generated from a highly homozygous accession.

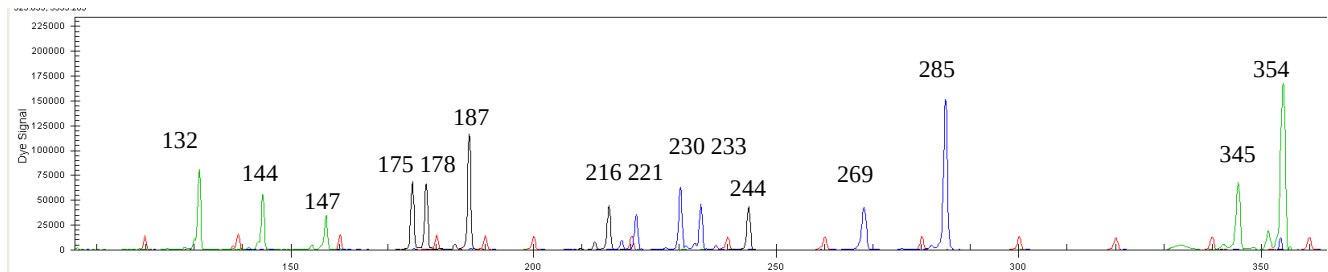
Projects in Progress in 2015

Black raspberry genomic resource development. We are planning to complete the assembly of draft genome of black raspberry and make it available to the scientific community in 2016. We are evaluating our populations for tolerance to *Verticillium dahliae* with the intent of placing a locus for this valuable trait on the linkage map. Additional aphid resistant crosses were generated evaluated for aphid resistance in a greenhouse setting. We have sequenced four populations with the different sources of resistance using the genotyping by sequencing (GBS) method that allows us to efficiently scan the genome for differences among the populations. We are in the process of fine mapping the region of linkage group 6 that is associated with aphid resistance to develop markers that can detect each of the sources of resistance and allow their use in breeding. We are also in the process of using the genetic linkage map to detect regions of the genome involved in controlling various traits of economic and breeder interest, such as flowering time, fruit chemistry, and plant vigor in different geographic locations. These genomic resources are essential for building the infrastructure needed for identification of candidate genes or closely linked markers for traits of interest during the development of improved black raspberry cultivars, and will inform decisions regarding germplasm value and usage, crossing, and selection through marker-assisted breeding.

Agarose gel image showing a DNA band that indicates the association of locus S525_109962 with aphid resistance (R) from Michigan (MI) and Ontario (ON) sources, but not with the Maine (ME) source or with susceptible (S) individuals.



Development of blackberry, pear, blueberry and hazelnut fingerprinting sets. A reliable and fast method for confirming identity and paternity in each of these crops is needed. Microsatellite or SSR markers are ideal for cultivar fingerprinting, paternity testing and identity certification. SSRs with higher core repeats have become available in each of these crops and are more reproducible across labs and easier to score than the previously used dinucleotide-containing SSRs. Our objective is to develop a multiplexed fingerprinting set composed of trinucleotide-containing SSRs that can distinguish each accession and use them to confirm identity in each of these crops.



Genetic profile of 'Black Satin' with the 6-SSR blackberry multiplex set indicating allele sizes in base pairs. Colors indicate fluorescent WellRED dyes used to label forward primers at each SSR: D2, black; D3, green; and D4, blue.

RosBREED: Combining disease resistance with horticultural quality in new rosaceous cultivars. We are in the process of optimizing a multiplexed fingerprinting set in each of blackberry and pear to use in parentage confirmation in pedigree-linked breeding populations in the University of Arkansas and Oregon USDA-ARS blackberry breeding programs and the USDA-ARS pear breeding program. We are in the process of evaluating OpenArrays and KASP markers for marker conversion of fire blight resistance SNP markers in pear. In strawberry, we plan on completing QTL analyses for remontancy, soluble solids content, fruit size, firmness, internal color, external color, pH and titratable acidity traits using phenotypic and genotypic data collected in the first RosBREED project.

Plant Pathology

By Joseph Postman

Farm Bill funds administered through APHIS were received to continue a survey of viruses in NCGR collections. Samantha Tochen was hired to conduct ELISAs.

- **Hazelnut (*Corylus*):** 846 trees were tested for Apple mosaic virus (ApMV) by ELISA. 5 trees in the field collection tested positive that were not previously known to be infected. This could be the result of pollen spread of ApMV within the collection, or infections that were missed in prior testing. The infected trees were removed from the field collection.
- **Rubus:** 907 clonal screenhouse accessions were tested by ELISA for ApMV, RBDV, TobRSV and TomRSV.
 - 44 tested positive for RBDV (Raspberry bushy dwarf virus) (4.9%) - 14 were new detections.
 - 2 tested positive for TomRSV (Tomato ringspot virus) (0.2%) - one was a new detection

- 1 tested positive for TobRSV (Tobacco ringspot virus). This is the first time this virus has been detected in Rubus at NCGR. The infected plant was a cultivar developed in Texas and received from a nursery in Michigan.
- ApMV was not detected in the Rubus collection.
- **Fragaria:** More than 900 accessions were tested by ELISA for ApMV, RpRSV, SLRSV, SMYEV, SNSV, and TomRSV.
 - 2 plants from the strawberry virus isolate collection tested positive for ApMV
 - No plants tested positive for RpRSV (Raspberry ringspot virus) or SLRSV (Strawberry latent ringspot virus).
 - More than 300 plants tested positive for SMYEV (Strawberry mild yellow edge virus). Many of these were new detections.
 - Only the positive control tested positive for TomRSV (Tomato ringspot virus)
 - 14 plants tested positive for SNSV (Strawberry necrotic shock virus). 4 of these were from the strawberry virus isolate collection.
- **Vaccinium:** 732 screenhouse plants were tested for BlScV, BlShV, BlMoV, BSSV, TobRSV, and TomRSV.
 - 2 cranberry accessions that had been received from southern Oregon tested positive for Blueberry scorch virus. This virus is not uncommon in cranberry fields in that area. The infected clones were duplicates of other accessions and were destroyed.
 - 1 blueberry screenhouse plant tested positive for Blueberry shock virus.
- **Vaccinium field survey** for Blueberry shock virus:
Plants in the USDA breeding program (as reported by Chad Finn) and at NCGR, have been tested regularly by ELISA for BlShV and germplasm that is particularly slow to become infected has been identified. At the OSU North Willamette Experiment Station ‘Legacy’, ‘Toro’, ‘Bluecrop’, ‘Baby Blues’, ‘Darrow’ and rabbiteye cultivars have tested negative for BlShV for over 10 years while growing among many known positive plants. At NCGR, ‘Bladen’, ‘Harding’, ‘Lateblue’, ‘Legacy’, ‘Razz’, US 612, US 693, US 845 and US 847 all tested negative after 20 years in the field. ‘Toro’ and ‘Bluecrop’, which had tested negative after 20+ years at NWREC, were positive at the NCGR, as were many rabbiteye cultivars.

Xylella fastidiosa detected in NCGR pears. In October, 2015, the presence of the bacterium *Xylella fastidiosa* was confirmed by the Oregon Department of Agriculture (ODA) in several pear trees growing in the Pyrus field germplasm collection. Preliminary DNA sequence data suggests it is the isolate *X. fastidiosa* subsp. *multiplex*, which can cause a chronic leaf-scorching disease in many different species of woody landscape shrubs and shade trees. This disease is widely distributed in the southern and eastern United States, where it is also known as ‘Oak Leaf Scorch’ or ‘Almond Leaf Scorch’, however *X. fastidiosa* is not known to be present in the state of Oregon and pear trees have not been reported as a host in North or South America. ODA imposed a state quarantine on the movement of Pyrus germplasm from the Repository, and NCGR suspended distribution of dormant Pyrus grafting wood during the winter of 2015-16. A survey will be conducted in summer 2016 to determine the distribution of the pathogen.

Publications

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3. **Bushakra, J.**, Bryant, D.W., Dossett, M., Vining, K.J., Vanburen, R., Gilmore, B.S., Lee, J., Mockler, T.C., Finn, C.E., **Bassil, N.V.** 2015. A genetic linkage map of black raspberry (*Rubus occidentalis*) and the mapping of Ag4 conferring resistance to the aphid *Amphorophora agathonica*. Theoretical and Applied Genetics. 128: 1631-1646.
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Presentations & Abstracts

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4. **Bushakra, J.**, Dossett, M., Lee, J.C., Lee, J., **Bassil, N.V.**, Finn, C.E. 2015. Molecular evaluation of aphid-resistant black raspberry germplasm for improved durability in black and red raspberry [abstract]. *American Society of Horticulture Science Meeting. 2015 American Society for Horticultural Science Annual Conference*.
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8. Ho, T., **Postman J.P.**, Martin, R.R. and Tzanetakis, I.E. Discovery, characterization and detection of five new virus species in *Ribes*. 2015 International conference on virus and other graft transmissible diseases of fruit crops, Morioka, Japan.
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